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The roles of TLRs, RLRs and NLRs in pathogen recognition

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REVIEW

Abstract

The mammalian innate immune system detects the presence of microbial infection through germ line-encoded pattern recognition receptors (PRRs). Toll-like receptors, retinoic acid-inducible gene-I-like receptors and nucleotide-binding oligomerization domain-like receptors serve as PRRs that recognize different but overlapping microbial components. They are expressed in different cellular compartments such as the cell surface, endosome, lysosome or cytoplasm and activate specific signaling pathways that lead to expression of genes that tailor immune responses to particular microbes. This review summarizes recent insights into pathogen sensing by these PRRs and their signaling pathways.

Introduction

The mammalian immune system consists of two different arms—innate and adaptive immunity—and cooperative interactions of these two arms are required for elimination of infective pathogens with the highest efficiency. The innate immune system is an evolutionarily conserved system that provides the first line of protection against invading microbial pathogens and is mediated by phagocytes such as macrophages and dendritic cells (DCs) (1–4). These cells sense microbial infection, engulf them and induce inflammatory responses. In contrast, adaptive immunity is highly specific and long lasting and has an immunological memory, but is initially developed in late phases of infection.

The specificity of the adaptive immunity relies on antigen-specific receptors expressed on the surface of T and B lymphocytes, which are generated as a consequence of gene rearrangements. Twenty years ago, Janeway proposed a hypothesis that the innate immune system senses microbial infection using receptors that are predominantly expressed on sentinel cells referred to as 'pattern recognition receptors (PRRs)' that recognize the molecular signature known as 'pathogen-associated molecular patterns (PAMPs)' (5). Because PAMPs are broadly expressed in pathogens but not in host cells, PRRs discriminate between self and non-self. In 1996, this hypothesis was supported by a study by Hoffmann's group, which demonstrated that mutant *Drosophila* carrying mutations in a receptor called 'Toll' exhibit high susceptibility to fungi infection owing to defective induction of anti-fungal peptides (6).

Subsequently, a human homologue of Toll (hToll)—now known to be Toll-like receptor (TLR) 4—was discovered and its ability to induce innate responses including production of inflammatory cytokines and expression of co-stimulatory molecules was demonstrated (7). A loss-of-function mutation of the mouse homologue of hToll was subsequently identified in mouse strains that are unable to promote innate immune responses against bacterial LPS (8–9). These earlier studies led to the identification of a family of membrane-bound TLRs (TLR1–TLR13), and mouse genetic studies revealed that TLRs generally serve as PRRs to recognize a wide range of PAMPs including lipids, lipoproteins, proteins, glycans and nucleic acids and play a central role in initiating innate immune responses (Table 1) (2).

Although the TLR family detects PAMPs either on the cell surface or the lumen of intracellular vesicles such as endosomes or lysosomes, recent studies have shown the existence of a cytosolic detection system for intracellular PAMPs. These cytosolic PRRs include retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). RLRs belong to the RNA helicases family that specifically detects RNA species derived from viruses in the cytoplasm (Table 1) and coordinate anti-viral programs via type I IFN induction (10). NLRs constitute a large family of intracellular PRRs, several of which—such as NOD1, NOD2 and NALP3 {NACHT [neuronal apoptosis inhibitory protein (NAIP), CIITA, HET-E and TP-1], LRR (leucine-rich repeat) and PYD (pyrin

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Table 1. PRRs and PAMPs

	PRRs (structure)	Adapters (structure)	PAMPs/Activators	Species
TLR	TLR1–TLR2 (LRR–TIR)	MyD88 (TIR–DD), TIRAP (TIR)	Triacyl lipopeptides	Bacteria
	TLR2–TLR6 (LRR–TIR)	MyD88 TIRAP	Diacyl lipopeptides	Mycoplasma
			LTA	Bacteria
			Zymosan	Fungus
	TLR2 (LRR–TIR)	MyD88, TIRAP	PGN	Bacteria
			Lipoarabinomannan	Mycobacteria
			Porins	Bacteria (*Neisseria*)
			tGPI-mucin	Parasites (*Trypanosoma*)
			HA protein	Virus (Measles virus)
	TLR3 (LRR–TIR)	TRIF (TIR)	dsRNA	Virus
	TLR4 (LRR–TIR)	MyD88, TIRAP, TRIF, TRAM (TIR)	LPS	Bacteria
			Envelope proteins	Virus (RSV, MMTV)
	TLR5 (LRR–TIR)	MyD88	Flagellin	Bacteria
	TLR7 (LRR–TIR)	MyD88	ssRNA	RNA virus
	hTLR8 (LRR–TIR)	MyD88	ssRNA	RNA virus
	TLR9 (LRR–TIR)	MyD88	CpG DNA	Bacteria
			DNA	DNA virus
			Malaria hemozoin	Parasites
	mTLR11 (LRR–TIR)	MyD88	Not determined	Bacteria (uropathogenic bacteria)
			Profilin-like molecule	Parasites (*Toxoplasma gondii)*
RLR	RIG-I (CARDx2–helicase)	IPS-1 (CARD)	RNA (5'-PPP ssRNA, short dsRNA)	Virus
	MDA5 (CARDx2–helicase)	IPS-1	RNA (poly IC, long dsRNA)	Virus
	LGP2 (helicase)		RNA	Virus
NLR	NOD1/NLRC1 (CARD–NBD–LRR)	RICK (CARD), CARD9 (CARD)	iE-DAP	Bacteria
	NOD2/NLRC2 (CARDx2–NBD–LRR)	RICK, CARD9	MDP	Bacteria
	NALP3/NLRP3 (PYD–NBD–LRR)	ASC (PYD–CARD) CARDINAL (PYD–FIND)	MDP	Bacteria
			RNA	Bacteria, Virus
			ATP	Bacteria? Host?
			Toxins	Bacteria
			Uric acid, CPPD, amyloid-β	Host
	NALP1/NLRP1 (CARD–FIND–NBD–LRR-PYD)	ASC	Anthrax lethal toxin	Bacteria
	IPAF/NLRC4 (CARD–NBD–LRR)		Flagellin	Bacteria
	NAIP5 (BIRx3–NBD–LRR)		Flagellin	Bacteria
CLR	Dectin-1 (lectin–ITAM)		β-Glucan	Fungi

domain) domains-containing protein 3}—are well characterized (Table 1) (11).

NOD1 and NOD2 recognize intracellular bacterial cell products, and NALP3 responds to multiple stimuli to form a multi-protein complex termed the NALP3 inflammasome, which promotes the release of the IL-1 family of cytokines (12–17). Furthermore, intracellular double-stranded DNA (dsDNA) released by DNA viruses or bacteria function as PAMPs that induce type I IFN through unidentified pathways (18, 19). In addition to PAMPs, innate immunity has the potential to respond to endogenous molecules that are released by host cells as a result of necrosis, pathogen infection, damage, injury and certain pathological conditions, which are directly or indirectly recognized by TLRs, NLRs, RLRs or as-yet-undefined sensors. The recognition of endogenous molecules by PRRs is tightly linked to the pathogenesis of autoimmune and inflammatory diseases.

In this review, we highlight recent advances in our understanding of innate immune recognition of PAMPs through TLRs, RLRs, cytosolic DNA sensors and NLRs. Next we describe the signaling pathways of these PRRs. Finally, we discuss the implications for adaptive immune responses and autoimmunity.

Structure of TLRs and their interactions with ligands

TLRs are type I transmembrane proteins (i.e. the N-terminal is outside the membrane) composed of three major domains and characterized by LRRs in the ectodomain, which mediate the recognition of their respective PAMPs; there is also a transmembrane domain and an intracellular domain that is homologous to that of the IL-1R and is known as the Toll/IL-1R (TIR) domain, which is required for initiating downstream signaling pathways. So far, the mammalian TLR family comprises more than 12 members (2). Although TLR1–TLR9 are conserved between humans and mice, TLR10 is not functional in mice because of a retrovirus insertion, and TLR11, TLR12 and TLR13 are lost in human genomes. The ligands for most TLRs were identified through generation of mice deficient for individual TLRs.

The LRR domain is composed of 19–25 tandem copies of LRR motifs, 20–30 amino acids in length, that contain the 'xLxxLxLxx' motif as well as 'xΦxxΦxxxxΦxxLx (Φ: hydrophobic)' sequences. Recent studies have revealed the crystal structure of TLR1, TLR2, TLR3 and TLR4 and suggest their mechanisms for recognizing cognate ligands (20). The LRR domain contains a β-strand and an α-helix linked by loops, which leads to the prediction that the LRR has a horseshoe-like structure. Viral double-stranded RNA (dsRNA), a TLR3 ligand, interacts with both the N-terminal and the C-terminal sites on the lateral side of the convex surface of TLR3 (Fig. 1G) (21). Ionic and hydrogen bonds with the sugar–phosphate backbones of dsRNA have been shown to contribute to the TLR3 interaction. On the other hand, bacterial lipopeptide, a ligand for the TLR1–TLR2 heterodimer, interacts with internal protein pockets, and hydrophobic interactions are responsible for recognition of the ligands (22) (Fig. 1H).

TLR4 is involved in recognition of bacterial LPS. TLR4 forms a complex with another LRR protein known as MD-2, and this is mediated by ionic and hydrogen bonds in two oppositely charged patches (23, 24). There is no direct interaction between TLR4 and LPS, but MD-2 functions as the LPS-binding component in the TLR4–MD-2 complex (Fig. 1A). Importantly, all these TLR ligands induce a homodimer or heterodimer of TLRs (TLR3–TLR3, TLR4–TLR4, TLR1–TLR2), all of which show the similar 'm'-shaped complexes. This dimerization is necessary for triggering downstream signaling by recruiting the TIR domain-containing adapter protein complex.

The TLR family members can be conveniently divided into two subpopulations with regard to their cellular localization. On the one hand, TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11 are expressed exclusively on the cell surface and recognize microbial membrane components such as lipids, lipoproteins and proteins. On the other hand, TLR3, TLR7,

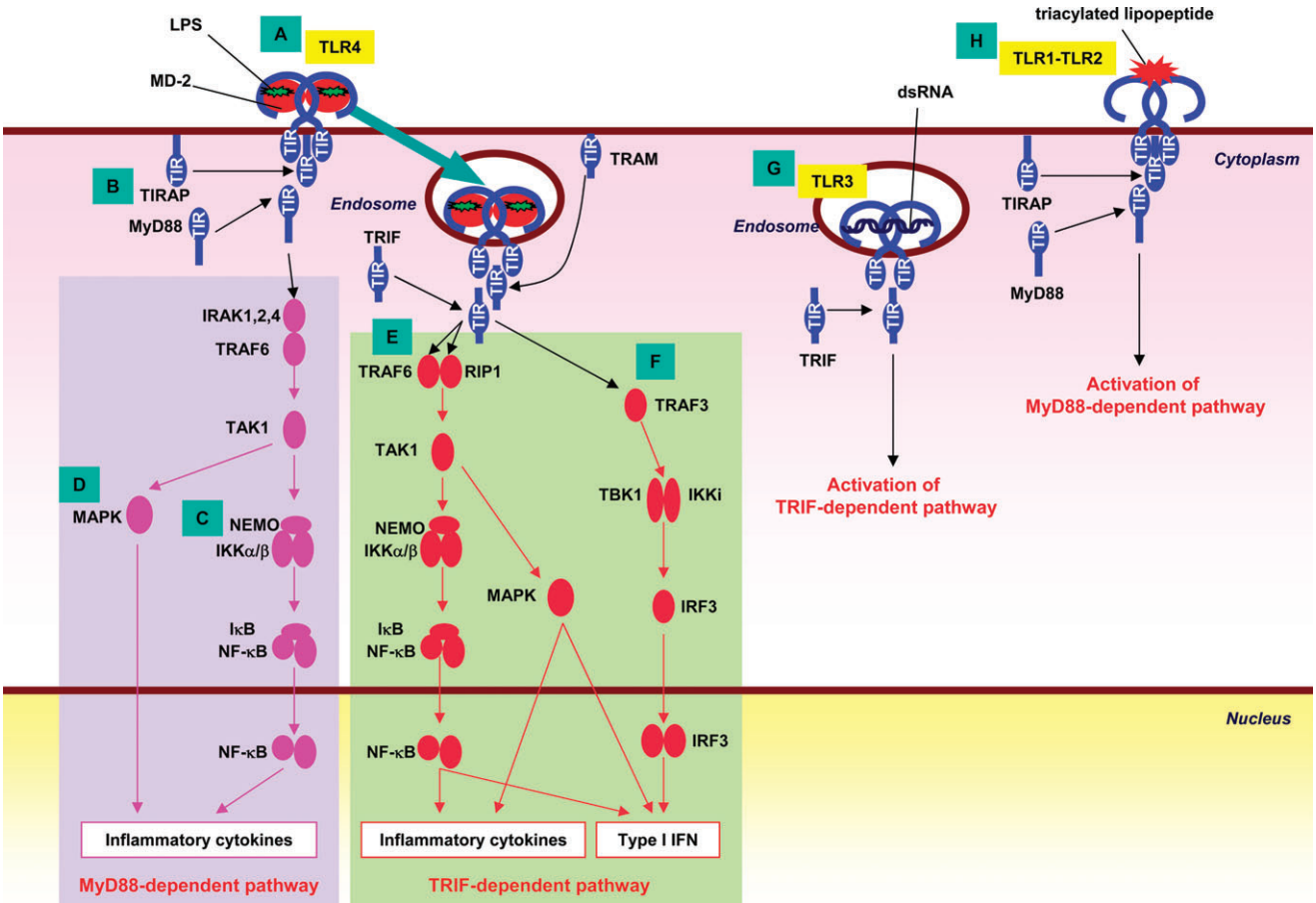

Fig. 1. Signaling pathways triggered by TLR3, TLR4 and TLR1–TLR2. (A) The TLR4–MD-2 complex engages with LPS on the cell surface via LBP and CD14 (data not shown) and then recruits a TIR domain-containing adapter complex including TIRAP and MyD88. The TLR4–MD-2–LPS complex is subsequently trafficked to the endosome, where it recruits TRAM and TRIF adapters. (B) TIRAP–MyD88 recruits IRAK family members and TRAF6 to activate TAK1. (C) The TAK1 complex activates the IKK complex composed of IKKα, IKKβ and NEMO (IKKγ), which catalyze phosphorylation of IκB proteins. Phosphorylated IκB proteins are degraded, allowing NF-κB to translocate to the nucleus. (D) TAK1 simultaneously activates the MAPK pathway. The activation of NF-κB and MAPK results in induction of inflammatory cytokine genes (MyD88-dependent pathway). TRAM–TRIF recruits (E) TRAF6 and RIP-1 for activation of TAK1 as well as (F) TRAF3 for activation of TBK1–IKKi that phosphorylates and activates IRF3. Whereas NF-κB and MAPK regulate expression of inflammatory cytokine genes in both pathways, IRF3 regulates expression of type I IFN in the TRIF-dependent pathway only. (G) TLR3 resides in the endosome and recognizes dsRNA. It recruits TRIF to activate the TRIF-dependent pathway. (H) TLR1–TLR2 recognizes bacterial triacylated lipopeptide and recruits TIRAP and MyD88 at the plasma membrane to activate the MyD88-dependent pathway.

TLR8 and TLR9 are localized in intracellular vesicles such as the endosome or lysosome and the endoplasmic reticulum (ER) and predominantly recognize microbial nucleic acid species.

Expression and ligands of cell-surface TLRs

TLR4 is essential for responses to LPS, a major constituent of the outer membrane of Gram-negative bacteria, which is a potent immunostimulatory molecule and causes septic shock. TLR4 is tightly associated with MD-2 on the cell surface and this complex is required for the robust induction of inflammatory cytokines (25). Additionally, LPS-binding protein (LBP) and CD14 are involved in the responses to LPS. LBP is present as a soluble protein or a plasma membrane protein and binds LPS. CD14, a glycosylphosphatidylinositol (GPI)-linked protein containing LRRs, binds LBP and delivers LPS–LBP to the TLR4–MD-2 complex (25). 'Smooth' LPS is composed of a polysaccharide O-antigen side chain and has complete core oligosaccharides, whereas 'rough' LPS lacks O-antigen and has shorter core oligosaccharides; both forms contain lipid A, a biologically active component of LPS. Cells lacking CD14 are unresponsive to smooth LPS; however, they still respond to rough LPS or lipid A. TLR4 is known to activate two signaling pathways—the myeloid differentiation primary response gene 88 (MyD88)-dependent pathway and the TIR-containing adapter inducing IFNβ (TRIF)-dependent pathway (Fig. 1)—whereas lipid A can signal only via the MyD88-dependent pathway in the absence of CD14 (26). These results suggest that the diversity of the structures of LPS among bacterial species may influence selective activation of these pathways. In addition to the detection of components of Gram-negative bacteria, TLR4 is implicated in the detection of envelope proteins of viruses such as respiratory syncytial virus (RSV) and mouse mammary tumor virus (2).

TLR2 recognizes a wide range of PAMPs derived from various pathogens, ranging from bacteria, fungi, parasites and viruses (2). These ligands include triacyl lipopeptides from bacteria and mycobacteria, diacyl lipopeptides from mycoplasma, peptidoglycan (PGN) and lipoteichoic acid (LTA) from Gram-positive bacteria, porin from *Neisseria*, lipoarabinomannan from mycobacteria, zymosan (containing β-glucan, mannans, chitin, lipid and protein) from fungi, *Trypanosoma* GPI-mucin (tGPI-mucin) and hemagglutinin protein from measles virus. TLR2 generally forms a heterodimer with TLR1, TLR6 or non-TLR molecules such as CD36, CD14 and dectin-1 to discriminate the molecular structure of the ligands. TLR2–TLR6 recognizes the mycobacterial diacylated lipopeptide, LTA and zymosan, whereas TLR2–TLR1 recognizes the bacterial triacylated lipopeptide. CD36, a member of a class II scavenger receptor expressed on the surface of innate immune cells, is critical in sensing some but not all TLR2 ligands, including TLR2–TLR6 ligands (27). CD14 is involved in recognition of diacylated lipopeptide and lipoarabinomannan. Dectin-1, an immunoreceptor tyrosine-based activation motif (ITAM)-containing C-type lectin receptor, binds β-glucan and induces its internalization; dectin-1 then collaborates with TLR2 to elicit inflammatory responses (28).

TLR5 recognizes flagellin, a protein component of bacterial flagella (29). It recognizes a highly conserved central site of flagellin, which is required for protofilament formation and bacterial motility. TLR5 was shown to be expressed on the basolateral surface of intestinal epithelial cells but not on macrophages or splenic DCs, suggesting a role of TLR5 in the detection of invasive flagellated bacteria in the gut. Subsequently, it was demonstrated that $CD11c^+$ $CD11b^+$ lamina propria dendritic cells (LPDCs) in the small intestine preferentially express TLR5 (30). LPDCs have the capacity to promote differentiation of T_h17 and T_h1 cells as well as differentiation of naive B cells into plasma cells for the production of IgA through TLR5. Notably, LPDCs, but not splenic DCs, have a unique property to produce retinoic acids, which control these humoral and cellular immune responses (31). Thus, TLR5 on LPDCs plays a critical role in regulating both innate and adaptive immune response in the intestine.

Mouse TLR11, which is a relative to TLR5, is highly expressed in the kidney and bladder. Accordingly, TLR11-deficient mice are susceptible to uropathogenic bacteria infection. Thus, TLR11 is likely to sense uropathogenic bacteria products although a ligand has not been identified yet (32). TLR11 also recognizes a parasite component. A soluble fraction of *Toxoplasma gondii* tachyzoites contains a potent inducer for IL-12 known as soluble *Toxoplasma* antigen. The active component is a profilin-like molecule that is known to function as an actin-binding protein and implicated in parasite motility or invasion (33). Mouse TLR11 recognizes the profilin-like molecule (34).

Of the cell-surface TLRs, TLR2 and TLR4 are also implicated in the recognition of endogenous molecules. These include heat shock proteins (HSP60, HSP70, gp96 and HSP22), fibrinogen, the extra domain A of fibronectins, hyaluronic acid, heparan sulfate, fatty acids, high-mobility group box 1 (HMGB1), modified low-density lipoprotein and β-defensin 2, most of which are released during inflammation or tissue damages or by necrotic cells. These endogenous ligands trigger production of TNFα, IL-12 and nitric oxide by macrophages. The role of these TLRs may be as sensors for danger signals (25).

Expression and ligands of intracellular TLRs

TLR3, TLR7, TLR8 and TLR9 are expressed by intracellular compartments such as the endosome, lysosome or the ER (35, 36) (Figs 1 and 2). These intracellular TLRs appear to be sensors of foreign nucleic acids and trigger anti-viral innate immune responses by producing type I IFN and inflammatory cytokines.

TLR3 recognizes a synthetic analogue of dsRNA polyinosinic-polycytidylic acid (poly IC), genomic RNA purified from dsRNA viruses such as reovirus and dsRNA produced during the course of replication of single-stranded RNA (ssRNA) viruses such as RSV, encephalomyocarditis virus (EMCV) and West Nile virus (WNV) (37, 38). Upon recognition of these RNA species, TLR3 is implicated in triggering anti-viral immune responses by producing type I IFN and inflammatory cytokines. Accordingly, TLR3-deficient mice died earlier than wild-type mice following infection with murine

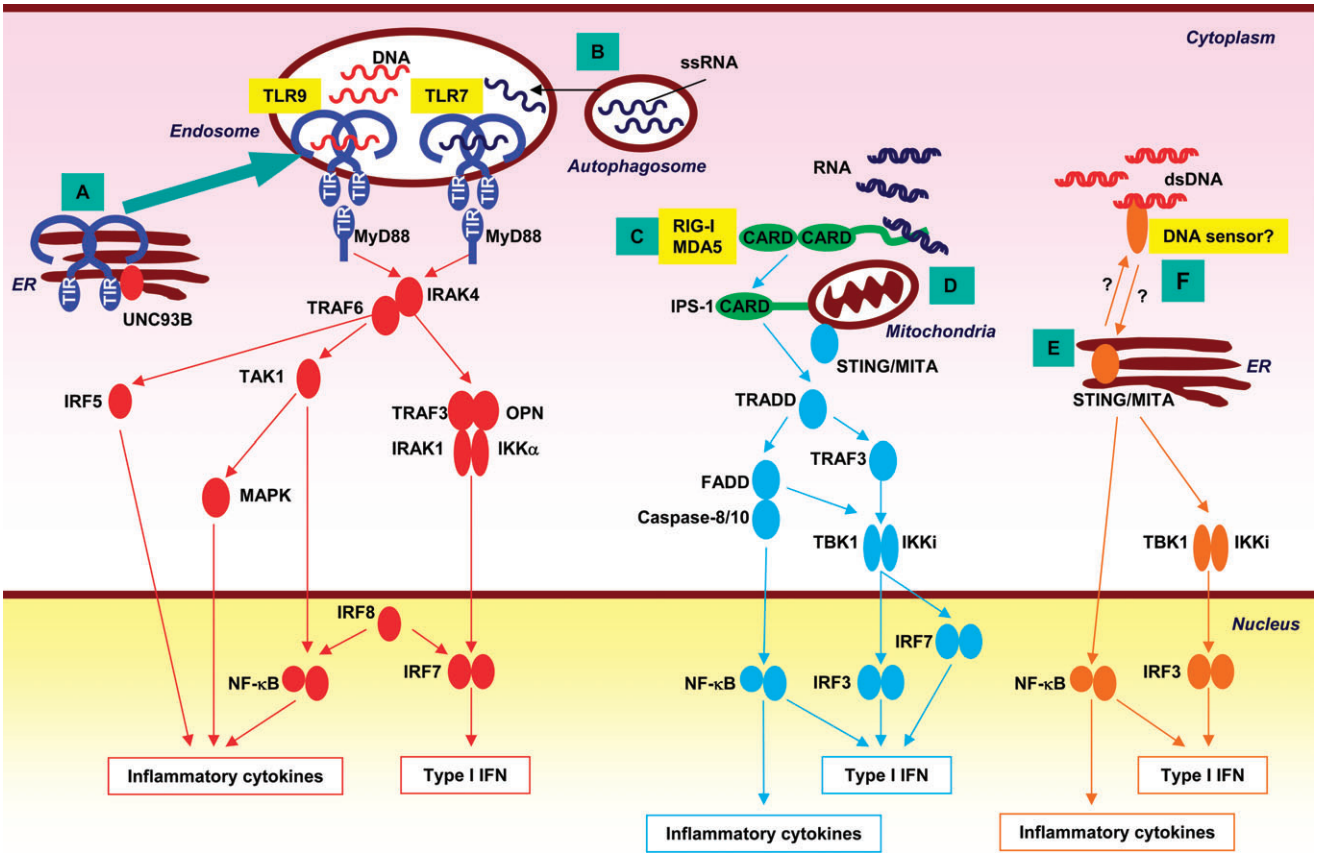

Fig. 2. Recognition of viral nucleic acids by TLRs, RLRs and the cytosolic DNA sensor. (A) In pDCs, TLR7 and TLR9 reside in the ER and interact with UNC93B and are trafficked to the endosome to recognize viral ssRNA and DNA, respectively. These TLRs recruit MyD88, IRAK4 and TRAF6, which in turn activates TAK1, IRF5 and TRAF3. TAK1 mediates activation of NF-κB and MAPK, which leads to the induction of inflammatory cytokine genes. IRF5 also mediates inflammatory cytokine expression. TRAF3 activates IRAK1 and IKKα, which catalyze the phosphorylation of IRF7 and induce type I IFN genes. OPN is involved in the activation of IRF7. IRF8 facilitates NF-κB and IRF7 activation. (B) In addition, pDCs exhibit constitutive autophagy induction, which deliver viral RNA to the endosome or lysosome, where TLR7 is expressed. (C) In cDCs, macrophages and fibroblast cells, viral RNA species are preferentially recognized by RLRs. RIG-I and MDA5 recruit the adapter IPS-1 via CARDs. IPS-1 is localized to mitochondria, and recruits TRADD, which then forms a complex with FADD, caspase-8 and caspase-10 to activate NF-κB. TRADD also recruits TRAF3 to activate the TBK1–IKKi–IRF3 axis. FADD is also implicated in IRF3 activation. STING (also known as MITA) localizes to (D) mitochondria or (E) ER; in mitochondria, STING (MITA) interacts with IPS-1 and RIG-I and activates NF-κB and IRF3. (F) Cytoplasmic dsDNA is thought to be sensed by an as-yet-undefined host DNA sensor. In the ER, STING (MITA) plays an essential role in the responses to dsDNA. DsDNA activates NF-κB and IRF3 via the IKK complex (data not shown) and TBK1–IKKi, respectively.

cytomegalovirus (MCMV), and TLR3 deficiency is associated with susceptibility to herpes simplex virus (HSV)-1 infection in humans (39, 40). There is, however, ample evidence for non-TLR3-dependent responses against viruses. TLR3-deficient mice mount $CD4^+$ and $CD8^+$ T cell responses against MCMV, vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV) and reovirus, similar to wild-type mice, and the susceptibilities to infection with these viruses were comparable (41); moreover, it was shown that TLR3-mediated recognition contributes to the pathogenesis rather than protection in the case of influenza A virus or WNV (38, 42). Collectively, although TLR3 recognizes dsRNA, it is not sufficient for anti-viral responses *in vivo*.

TLR3 messenger RNA (mRNA) is detected in conventional dendritic cells (cDCs) and macrophages as well as by non-immune cells including fibroblasts and epithelial cells, and strong expression of TLR3 is found in $CD8\alpha^+$ DCs with high phagocytic activity for apoptotic bodies of virus-infected or dsRNA-loaded cells. This allows dsRNA to gain access to TLR3 within cells and activate the signaling cascade to produce IL-12 p40 and IFNβ, suggesting a role of TLR3 in triggering cross-presentation, which processes exogenous antigens within the MHC class I pathway (43).

TLR3 is also implicated in the recognition of small interfering RNA (siRNA). TLR3 recognizes siRNA in a sequence-independent manner and induces the production of IL-12 and IFNγ, which efficiently suppress angiogenesis in a mouse model of choroidal neovascularization, indicating that siRNA-induced, TLR3-mediated innate immune responses, rather than suppression of gene expression, are important for the inhibition of angiogenesis (44).

TLR7 was originally identified to recognize imidazoquinoline derivatives such as imiquimod and resiquimod (R-848) and guanine analogues such as loxoribine, all of which have anti-viral and anti-tumor properties (45). Guanosine-rich and uridine-rich ssRNA derived from HIV or influenza virus, synthetic polyuridine ssRNA and certain siRNAs were subsequently identified as ligands for TLR7 (46, 47). TLR7 is

highly expressed on plasmacytoid dendritic cells (pDCs), a subset of DCs with a plasmacytoid morphology that are unique in their capacity to rapidly secrete vast amounts of type I IFN in response to viral infection (48). Accordingly, type I IFN production in response to infection with influenza virus or VSV was abrogated in TLR7-deficient pDCs (46, 49). It was shown that the induction of type I IFN by pDCs occurs independently of replication of enveloped viruses, including influenza and herpes viruses. These viruses are likely to be endocytosed and retained in the endosomal compartments, where the viral particles are subsequently degraded, allowing the viral RNA to engage with TLR7.

TLR8 is phylogenetically the most similar to TLR7. Human TLR8 preferentially recognizes R-848, bacterial RNA and ssRNA derived from HIV, VSV and influenza A virus, although TLR8-deficient mice respond normally to these molecules, suggesting a species-specific function of TLR8 (47). TLR8 is expressed in various tissues, with the highest expression in monocytes, and is up-regulated upon bacteria infection.

TLR9 was originally identified to recognize unmethylated 2′-deoxyribo cytidine–phosphate–guanosine (CpG) DNA motifs that are frequently present in bacteria, but are rare in vertebrates (50). Synthetic CpG oligodeoxynucleotides (ODNs) function as TLR9 ligands, and TLR9 recognition of DNA occurs independently of the base sequence. The sugar backbone 2′-deoxyribose of DNA is sufficient to confer signaling (51). Given that pDCs produce vast amounts of type I IFN in response to DNA virus infection or certain CpG ODNs, TLR9 expressed by pDCs may serve as a sensor for virus infection. Consistently, IFNα production following infection with DNA viruses, such as MCMV, HSV-1 and HSV-2, is totally dependent on TLR9 in pDCs (52–54). In addition to DNA, hemozoin (Hz) derived from *Plasmodium falciparum* potently activates macrophages and DCs to produce inflammatory cytokines and chemokines through TLR9 (55–57). Hz is an insoluble crystal generated as a by-product of the detoxification process after parasite digestion of host hemoglobin. TLR9-deficient mice display partial resistance against lethal infection of the rodent malaria parasite *Plasmodium yoelii* owing to reduced regulatory T cell activation (58). Thus, malaria parasites may target TLR9 as an evasion mechanism.

Trafficking of intracellular TLRs

The intracellular TLRs, including TLR3, TLR7, TLR8 and TLR9, are expressed on the ER in resting cells and trafficked to the endosomal compartment in response to PAMP-mediated stimulation. This intracellular localization is important for the recognition of viral nucleic acids that are delivered to TLR-expressing intracellular vesicles through the endosomal pathway; moreover, this is also important for discrimination of self from non-self nucleic acids since ectopic expression of TLR9 on the macrophage cell surface causes it to respond to DNA derived from self (59).

The intracellular localization of nucleic acid-sensing TLRs is controlled by UNC93B, a 12-membrane-spanning ER protein. Mice bearing a single missense mutation in the gene encoding UNC93B have defects in cytokine production as well as up-regulation of co-stimulatory molecules in response to TLR3, TLR7 and TLR9 ligands (60). UNC93B interacts with the transmembrane regions of TLR3, TLR7 and TLR9 in the ER and assists in the trafficking of TLR7 and TLR9 from the ER to the endosome (61, 62) (Fig. 2A). It has recently been proposed that proteolysis of TLR9 within the endolysosomal compartment is essential for robust innate immune responses (63, 64). The ectodomain of TLR9 is cleaved by cathepsins, and the cleaved product can activate downstream signaling.

As mentioned above, TLR7 sensing of viral ssRNA within the endosome is replication independent. It can, however, also sense replicating VSV that enter the cytoplasm. It was demonstrated that autophagy, a process for lysosomal degradation of cellular organelles or pathogens, mediates the delivery of cytosolic viral replication intermediates to the lysosome, where TLR7-mediated recognition occurs (Fig. 2B). Accordingly, pDCs derived from mice deficient in autophagy-related 5 homologue (ATG5), which is required for autophagosome formation, failed to produce IFNα following VSV infection (65); moreover, autophagosome formation occurs constitutively in pDCs. These findings indicate that autophagy is an essential mechanism for sensing RNA viruses in pDCs. Roles of ATG5 in facilitating innate immune responses were also suggested in macrophages. When engulfed by macrophages, TLR ligand-conjugated particles trigger the recruitment of light chain 3 (LC3), a marker of autophagosomes, to phagosomes in a manner dependent on ATG5, which results in rapid acidification and enhanced macrophage killing activity (66); however, in fibroblast cells, ATG5 has a role in suppressing anti-viral innate immune responses by inhibiting RLR signaling (67). Further work will be required to reveal mechanisms of cell-type-specific function of ATG5 in host defense.

Structure and ligands of the RLR family

Once viruses enter the cytoplasm and generate dsRNA during the course of replication, infected host cells can sense them and, thus, activate intrinsic anti-viral signaling pathways. This sensing occurs in the cytoplasm of both immune and non-immune cells and is independent of the TLRs that can detect the RNA species present within endosome (68). In this regard, the RLR family, which has three members—RIG-I, melanoma differentiation associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2)—was reported to recognize viral RNA in the cytoplasm (2, 10) (Fig. 2C).

RIG-I, a prototypical member of the RLR family, contains tandem caspase recruitment domain (CARD)-like regions at its N-terminus that function as an interaction domain with other CARD-containing proteins, the central the DExD/H helicase domain, which has an ATP-binding motif. RIG-I also has a C-terminal repressor domain (RD), which binds to RNA (69, 70). In resting cells, RIG-I is inactive as a monomer, but virus infection and RNA binding trigger conformational changes to facilitate self-association, which promotes CARD interaction with downstream signaling molecules. MDA5 contains tandem CARD-like regions and a DExD/H helicase domain, but it is unknown whether the C-terminal region of MDA5 really functions as an RD. LGP2 contains a DExD/H helicase domain and an RD, but lacks the CARD-like region. LGP2 was suggested to serve as a negative regulator of

RNA virus-induced responses, because the LGP2 RD binds to that of RIG-I and suppresses signaling by interfering with the self-association of RIG-I (10).

The roles of RLRs in the detection of RNA viruses have been elucidated through analyses of mice deficient for each respective RLR (71, 72). RIG-I is essential for the recognition of various ssRNA viruses, which include paramyxoviruses, influenza A virus, VSV and Japanese encephalitis virus. MDA5 is required for the recognition of other RNA viruses, including picornaviruses such as EMCV, Mengo virus and Theiler's virus; moreover, MDA5 is involved in the recognition of poly IC. Mice deficient for RIG-I and MDA5 are consistently more susceptible to infection with the respective viruses than wild-type mice are.

These findings suggest that RIG-I and MDA5 have specificities in their detection of RNA viruses, presumably through recognition of distinct structures of viral RNA. This is likely because RIG-I is activated following transfection of *in vitro* transcribed RNA, whereas MDA5 is activated by poly IC. RIG-I was demonstrated to recognize ssRNA bearing a 5′-triphosphate moiety (73, 74). Accordingly, the induction of anti-viral responses following infection with influenza A viruses, which contain 5′-triphosphate structures but create little dsRNA, is controlled by RIG-I. Notably, 5′-triphosphate structures are removed or masked by a cap structure in the case of self-RNA, which suggests a discrimination mechanism between self- and non-self RNA; however, the 5′-triphosphate structure is necessary but not sufficient for RIG-I recognition. Rather, RIG-I recognition is determined by a homopolymeric ribonucleotide composition such as the polyuridine motif of the hepatitis C virus (HCV) genome 3′-non-translated region, by linear RNA structure and by RNA length (75); moreover, it was shown that RIG-I recognizes small dsRNA species ranging from 21 to 27 nucleotides without a 3′-overhang (76), and RIG-I and MDA5 distinguish dsRNA by size; RIG-I binds short dsRNA, whereas MDA5 binds long dsRNA (77).

Despite the initial implication as a negative regulator, LGP2-deficient mice exhibit complicated phenotypes (78). They show elevated levels of type I IFN in response to poly IC and VSV, but decreased type I IFN following EMCV infection, suggesting that LGP2 negatively or positively regulates RIG-I and MDA5 responses, depending on the type of RNA viruses.

An as-yet-undefined cytosolic DNA sensor

When released into the cytoplasm, dsDNA also has properties that promote anti-viral and inflammatory responses. This response occurs after infection with DNA viruses or certain bacteria through TLR9-independent and RLR-independent pathways (18, 19). Similar to RLRs, this recognition occurs in the cytoplasm of many cell types including immune and non-immune cells and triggers a type I IFN response via TBK1 [TNFR-associated factor (TRAF) family member-associated nuclear factor κB (NF-κB) activator (TANK)-binding kinase 1], a protein kinase that phosphorylates the transcription factor IFN regulatory factor (IRF) 3 (Fig. 2F) (79, 80). This is in contrast to endosome-localized TLR9, which functions in DCs and B cells and triggers type I IFN without the need for TBK1 (81). Whereas right-hand B-form dsDNA shows high immunostimulatory activity with regard to cytokine induction, the left-hand Z-form dsDNA or ssDNA has low or no activity (79).

Although little is known about the recognition mechanism of cytoplasmic DNA, DNA-dependent activator of IRF (DAI, also known as Z-DNA-binding protein 1 and DLM1) has been isolated as a DNA sensor with DNA-binding and TBK1-activating properties (82); however, responses against dsDNA were unaffected by DAI deficiency in mice, suggesting a redundant or non-essential role of DAI (83).

Recently, stimulator of IFN genes (STING), a membrane protein predominantly expressed in the ER, has been identified as a molecule whose over-expression significantly activates the IFNβ promoter (84) (Fig. 2D and E). STING over-expression up-regulates type I IFN genes and suppresses viral replication. STING-deficient cells showed diminished type I IFN induction following cytosolic dsDNA stimulation or following infection with *Listeria monocytogenes* or HSV-1, in which DNA is responsible for activating the host innate responses. STING has been shown to physically interact with β-signal sequence receptor gene [SSR2, also known as translocon-associated protein (TRAP) β], a subunit of the TRAP complex, which mediates the translocation of nascent polypeptides into the lumen of the ER. This interaction is required for STING's ability to induce type I IFN (84). Collectively, these findings suggest that STING may couple a DNA sensor to the TRAP complex, which is a prerequisite to activating the IFNβ promoter. Alternatively, STING may participate in ER stress responses, which may occur following DNA stimulation, although it is not clear whether ER stress is linked to anti-viral responses.

The structure and phylogeny of the NLR family

The NLR family detects the presence of PAMPs and endogenous molecules in the cytosol (12–17). NLRs consist of three domains characterized by an N-terminal protein interaction domain, a central nucleotide-binding domain and a C-terminal LRR. Members of the NLR family are categorized into at least five subfamilies distinguished by their N-terminal structures. These include NLRA (which contain an acidic transactivation domain), NLRB (contain a baculovirus inhibitor of apoptosis protein repeat [BIR]), NLRC (contain a CARD), NLRP (contain a Pyrin domain) and NLRX (contain an unknown domain). So far, at least 23 human and 34 murine NLR genes have been identified, although the physiological function of most NLRs is poorly understood (11).

NLR orthologues are present in plant R genes. In plants, which lack adaptive immunity, host defense solely relies on the innate immune system, and detection of pathogens is mediated by hundreds of R proteins. Notably, pathogen detection in plants occurs through an indirect interaction in which R proteins interact with intermediates of host cells that are modified during a course of infection (this is part of the 'guard hypothesis') (85). This suggests the possibility that NLRs indirectly sense PAMPs; moreover, the nucleotide-binding site–LRR sequences that are characteristic of R proteins and the mammalian NLRs constitute similar protein complexes. R proteins form a complex along with suppressor of G_2 allele of S-phase kinase-associated protein 1 (SGT1) and HSP90, which is required for R protein stability and activation of signaling, and indeed, several mammalian NLRs, including NOD2, IL-1β-converting enzyme protease-activating factor

(IPAF), NALP3 and Monarch-1 (NLRP12), form a pre-activation complex with homologues of SGT1 and HSP90 (86, 87).

Ligands of NOD1 and NOD2

NOD1 (which is categorized as NLRC1) and NOD2 (NLRC2) are well-characterized members of the NLR family, which recognize distinct structural motifs derived from PGN. NOD1 recognizes g-D-glutamyl-meso-diaminopimelic acid (iE-DAP), which is found in the PGN structures of all Gram-negative as well as in several Gram-positive bacteria such as *Bacillus subtilis* and *L. monocytogenes* (88, 89). In contrast, NOD2 recognizes muramyl dipeptide (MDP), the largest component of the PGN motif that is also present in all Gram-negative and Gram-positive bacteria (90, 91) (Fig. 3).

NOD1 is implicated in the intracellular recognition of various pathogenic bacteria such as enteroinvasive *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Chlamydia species*, *Campylobacter jejuni*, *Haemophilus influenzae* and *Helicobacter pylori* (16). NOD2 participates in sensing *Streptococcus pneumonia* and *Mycobacterium tuberculosis* (16). *Listeria monocytogenes* activates both NOD1 and NOD2. Signaling via NOD1 and NOD2 results in the induction of inflammatory cytokines and other anti-microbial genes, which contributes to host defense (92, 93). The mechanisms by which PGN is delivered into the cytosol to gain access to NOD1 and NOD2 are unclear. It was shown that *H. pylori* introduces iE-DAP into the cytosol using its type IV secretion system; iE-DAP in turn engages with NOD1 (92).

Types of inflammasome

Certain NLRs respond to many PAMPs and lead to the release of the IL-1 family of inflammatory cytokines including

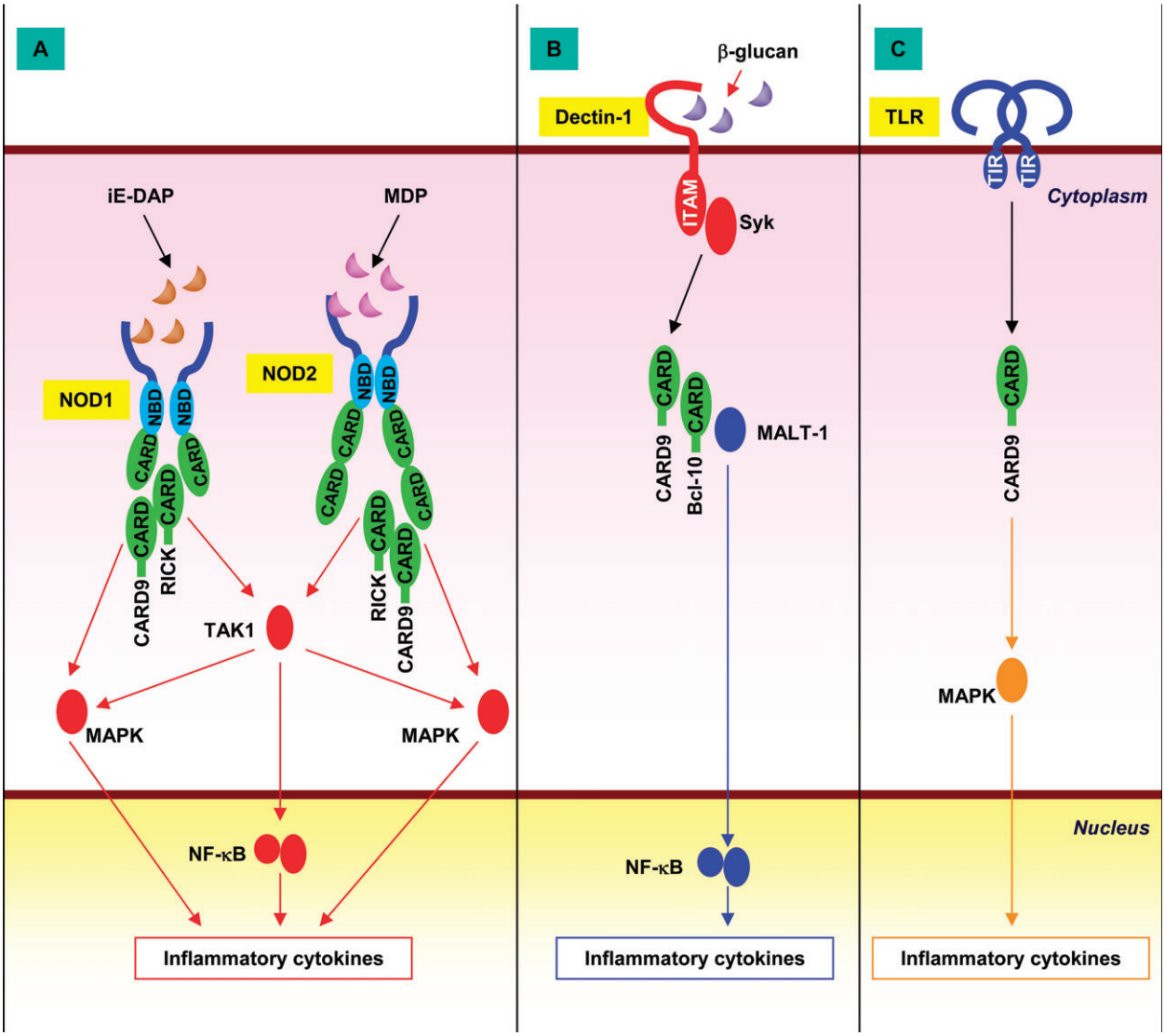

Fig. 3. Sensing PAMPs by NOD1, NOD2, dectin-1 and TLRs. (A) NOD1 and NOD2 sense intracellular iE-DAP and MDP, respectively, and recruit CARD proteins RICK and CARD9. RICK activates MAPK and NF-κB via TAK1; CARD9 activates MAPK. (B) Dectin-1 senses fungal infection and recruits the Syk, which leads to activation of NF-κB through the CARD9–Bcl-10–MALT1 complex. (C) CARD9 is also involved in TLR-mediated MAPK activation. Activation of NF-κB and MAPK results in induction of inflammatory cytokine genes.

IL-1β, IL-18 and IL-33 through the formation of the 'inflammasome', which involves caspase-1 (13–17). Caspase-1 mediates the processing of the pro-form of these cytokines into mature forms, which results in the secretion of bioactive cytokines. On the basis of the NLR protein involved, inflammasomes are grouped into three main types—the NALP3 inflammasome (also known as the NLRP3 inflammasome), the NALP1 (NLRP1) inflammasome and the IPAF (NLRC4) inflammasome (Fig. 4). These inflammasomes involve an adapter—apoptosis-associated speck-like protein containing a CARD (ASC)—that links these NLRs to caspase-1. Additionally, CARDINAL (CARD8, DACAR, NDPP1 and TUCAN) is involved in the NALR3 inflammasome (Fig. 4A).

Triggers for the NALP3 inflammasome

The formation of the NALP3 inflammasome is triggered by various PAMPs with diverse structures. NALP3 is required for caspase-1 activation in response to TLR ligands such as LPS, dsRNA, PGN and LTAs when stimulated together with extracellular ATP (94–97). Bacterial RNA or a synthetic imidazoquinoline-like compound (R837 or R848) alone is sufficient to activate caspase-1 through NALP3, but ATP co-stimulation enhances the activation (96) (Fig. 4).

Thus, these PAMPs prime cells to induce synthesis of pro-IL-1β, and subsequent exposure to ATP potently triggers caspase-1 activation to promote processing of pro-IL-1β and the release of IL-1β. ATP, which is released by pathogens, necrotic damaged cells or TLR ligand-stimulated monocytes (98, 99), activates the purinergic P2X7 receptor that recruits the hemichannel protein pannexin-1, which activates caspase-1 through NALP3 (100–102) (Fig. 4B). Although the mechanisms of pannexin-1-triggered NALP3 inflammasome activation are unclear, it was proposed that P2X7-dependent activation of pannexin-1 mediates delivery of PAMPs into the cytoplasm, where the NALP3 inflammasome is formed; however, no direct interaction between PAMPs and NALP3 has been demonstrated, suggesting that the PAMP recognition through NALP3 is indirect, similar to that in plant R proteins. It was shown that stimulation of the P2X7 receptor with ATP induces K^+ efflux, which is important

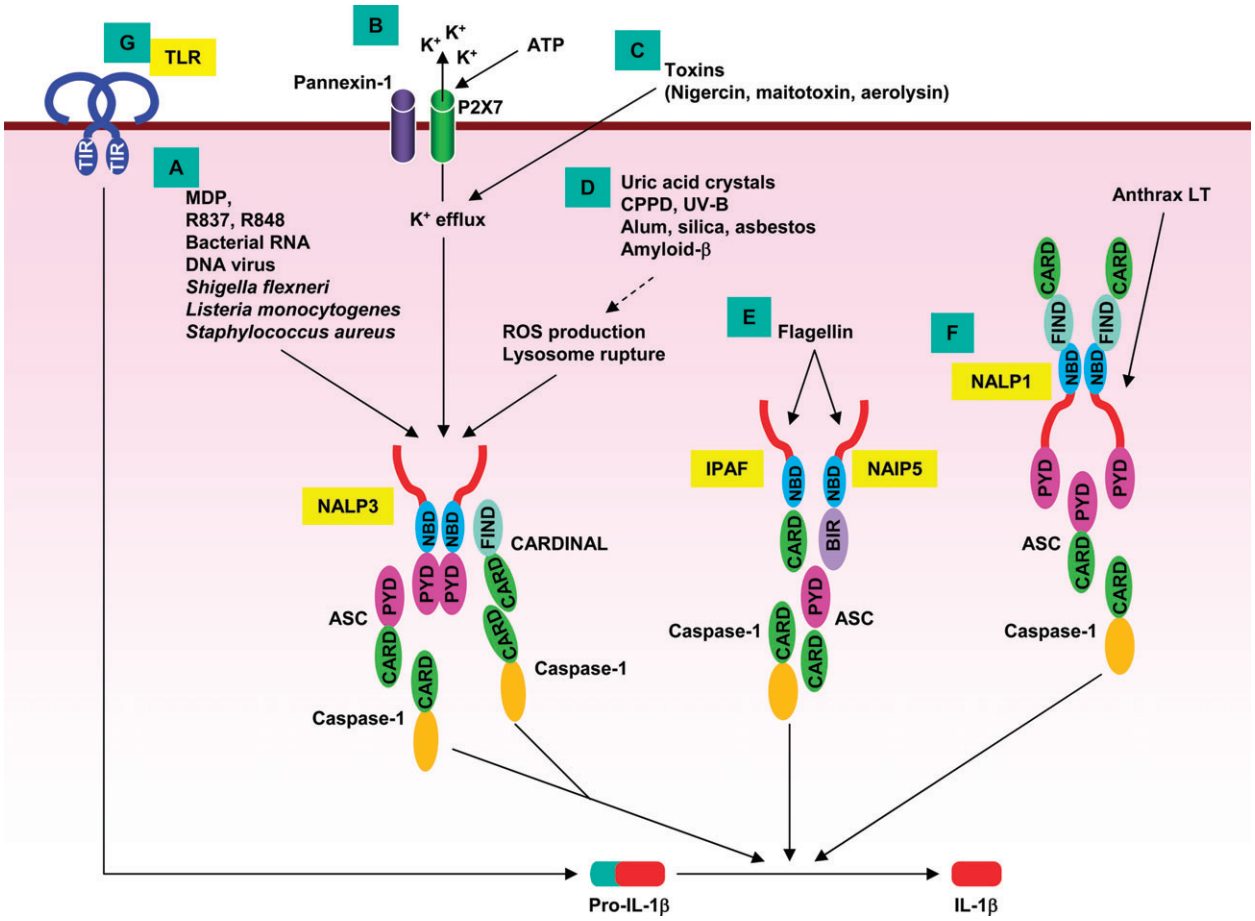

Fig. 4. Activation of IL-1β by inflammasomes and other pathways. The three types of inflammasome shown here can recruit caspase-1, which converts pro-IL-1β into the mature form, IL-1β. (A) MDP, R837, R848, bacterial RNA, DNA viruses and several intracellular bacteria activate the NALP3 inflammasome. NALP3 forms a complex with the adapters ASC and CARDINAL to recruit caspase-1. (B) Extracellular ATP activates the P2X7 purine receptor, which then helps to allow the pannexin-1 receptor to cause K^+ efflux, which enhances activation of the NALP3 (and NALP1) inflammasome. (C) Some microbial toxins, and (D) gout-associated uric acid crystals, calcium pyrophosphate dihydrate crystals (CPPD), UV-B irradiation, alum, silica, asbestos and amyloid-β also induce activation of the NALP3 inflammasome. These stimuli induce ROS production or release of cathepsins during lysosome rupture. (E) Flagellin activates IPAF and NAIP5. The IPAF inflammasome recruits ASC and caspase-1. (F) The NALP1 inflammasome senses *Bacillus anthrax* LT and activates caspase-1 via ASC. (G) TLR ligands also induce the synthesis of pro-IL-1β.

for NALP3 (and NALP1) inflammasome activation (103, 104) (Fig. 4). Toxins such as nigericin (*Streptomyces hygroscopicus*), aerolysin (*Aeromonas hydrophila*), maitotoxin (*Marina dinoflagellates*), gramicidin (*Bacillus brevis*) and α-toxin (*Staphylococcus aureus*) potently activate NALP3, perhaps, through K^+ efflux (94, 105) (Fig. 4).

In addition, the NALP3 inflammasome is required for IL-1β release in response to MDP, pathogens (DNA viruses, RNA viruses, intracellular bacteria), non-PAMP crystals [silica, asbestos, aluminum salt (alum)], endogenous molecules (gout-associated uric acid crystals, calcium pyrophosphate dihydrate crystals, fibrillar peptide amyloid-β) and stresses (UV-B irradiation) (94, 106–113) (Fig. 4D). It was proposed that activation of the P2X7 receptor by ATP causes production of reactive oxygen species (ROS), which activate the inflammasome (Fig. 4D). Accordingly, ROS inhibitors abrogate the activation of the NALP3 inflammasome formed by asbestos, silica and alum (108, 114). Similarly, the release of cathepsin B from the lysosome after exposure to silica, alum or amyloid-β was shown to activate the NALP3 inflammasome (110, 112) (Fig. 4). Collectively, inflammasome activation is triggered by stress-induced or infection-induced intermediates including K^+ efflux, ROS species and cathepsins, and hence, the inflammasome may serve as a general sensor for cellular stresses.

Triggers and inhibitors of the NALP1 inflammasome

Bacillus anthrax lethal toxin (LT) is a potent toxin, which induces macrophage death. LT is composed of a protective antigen and lethal factors. The protective antigen binds to a cell-surface receptor to mediate the delivery of lethal factors to the cytosol of infected cells. LT-induced macrophage death requires caspase-1 (Fig. 4F). NALP1b alleles were identified within the locus responsible for susceptibility to LT (115); moreover, *B. anthrax*-induced IL-1β release is dependent on LT, suggesting that the NALP1 inflammasome is required for *B. anthrax*-induced IL-1β production. In addition, NOD2 is required for *B. anthrax*-induced IL-1β release, and NOD2–NALP1 protein complex are found in single inflammasomes in response to infection (116).

NALP1 was shown to form a complex with anti-apoptotic proteins B cell lymphoma (Bcl)-2 and Bcl-XL, which prevent NALP1-mediated caspase-1 activation, suggesting a role of Bcl-2 family members in inhibiting LT-induced cytotoxicity (117).

Triggers of the IPAF inflammasome

The IPAF inflammasome is activated by flagellin, which is delivered to the cytosol (118, 119) (Fig. 4E). Macrophages deficient in IPAF fail to activate caspase-1 and release IL-1β in response to intracellular bacteria such as *Salmonella typhimurium* and *Legionella pneumophila*, and flagellin-deficient mutants of these bacteria fail to activate the IPAF inflammasome (94, 118–121).

When flagellin is delivered to the cytoplasm through a pore-forming toxin such as listeriolysin O or a transfection reagent, it promotes the IPAF inflammasome; moreover, during infection with *S. typhimurium* and *L. pneumophila*, caspase-1 activation depends on functional type III and type IV secretion systems, respectively, suggesting that flagellin that leaks into the cytoplasm is responsible for the IPAF inflammasome activation. Macrophages lacking IPAF and caspase-1 have defects in fusion of *L. pneumophila*-containing phagosomes with lysosomes, and are more susceptible to infection, indicating the importance of the IPAF inflammasome in host defense (122); however, it has been suggested that IPAF senses PAMPs other than flagellin because flagellin-deficient bacteria or a pore-forming toxin are still capable of activating caspase-1 via IPAF (97, 105, 119). Given that ATP and K^+ efflux are not required for IPAF-mediated triggering of caspase-1 activation, IPAF may sense a wide variety of PAMPs in addition to flagellin that leaks into the cytosol through type III and IV secretion systems or via membrane pores.

Neuronal apoptosis inhibitory protein 5 [NAIP5, also known as BIR-containing 1e (Birc1e) and NLRB] also participates in the recognition of flagellin from *L. pneumophila* (121–123) (Fig. 4E). IPAF and NAIP5 bind to each other, suggesting that these proteins may act in concert to detect the presence of flagellin and elicit effective immune responses (122). It has been recently demonstrated that a 35-amino acid region of the C-terminus of flagellin, which is distinct from the TLR5 recognition site, is critical for IPAF-dependent caspase-1 activation and IL-1β release. NAIP5-deficient mice fail to respond to the 35-amino acid sequence of flagellin and fail to activate casapse-1 and, thus, show increased *L. pneumophila* replication (124). These results suggest that IPAF may form an inflammasome with many distinct proteins to recognize a wide variety of PAMPs, with IPAF–NAIP5 being restricted to flagellin recognition.

Signaling pathways of TLRs, RLRs and several NLRs converge on NF-κB and mitogen-activated protein kinase

Signaling pathways via TLRs, RLRs, NOD1 and NOD2 culminate in the activation of NF-κB and/or mitogen-activated protein kinases (MAPKs), which regulate the expression of numerous immune and inflammatory genes (2, 68, 125). The NF-κB family consists of five members that can exist as dimers and the heterodimer composed of RelA and p50 is considered to be the most frequently activated during PRR signaling. The transcriptional activation of NF-κB is controlled by multiple nuclear proteins. A member of evolutionally conserved akirin proteins, akirin2, contributes to the induction of certain NF-κB target inflammatory genes probably through modifying chromatin-remodeling proteins (126). A nuclear inhibitor of κB (IκB) protein, IκBζ, which is induced in a MyD88-dependent manner, physically interacts with the p50 subunit of NF-κB and accelerates the transcription of IL-6 (127). MAPKs include extracellular signal-regulated kinase 1/2 (ERK1/2), p38 and c-jun N-terminal kinases (JNKs), which phosphorylate the activator protein 1 family of transcription factors to regulate transcription or mRNA stability of inflammatory cytokine genes.

Several TLRs and RLRs that recognize viral PAMPs also activate members of the IRF family of transcription factors, which induce the expression of type I IFN and inflammatory genes (68). Below, we detail the molecules involved in IRF activation, but first, we describe the adapters and kinases

immediately downstream of TLRs; we focus on the many new components and regulatory mechanisms that have been defined in recent years.

Functional properties of adapters in TLR signaling

TLR signaling is initiated by the ectodomain-mediated dimerization of TLRs, which then facilitates the recruitment of TIR domain-containing cytosolic adapter molecules, including four in particular—MyD88, TIRAP (also known as MyD88 adapter like [MAL]), TRIF [also known as TIR domain-containing adapter molecule (TICAM) 1] and TRIF-related adapter molecule (TRAM, also known as TICAM-2)—to the receptor complex (2, 128) (Figs 1 and 2). These adapters are selectively recruited to their respective TLRs, eliciting appropriate responses depending on the type of PAMP. MyD88 is utilized by all TLRs with the exception of TLR3 and drives NF-κB and MAPK activation to control inflammatory responses. TIRAP is recruited to TLR2 and TLR4 and functions as a sorting adapter that recruits MyD88. TRIF, in contrast, is used by TLR3 and TLR4 and initiates an alternative pathway leading to IRF3, NF-κB and MAPK to induce type I IFN and inflammatory cytokines. TRAM selectively serves to link TRIF to TLR4, but not TLR3 (Fig. 1).

TLR4, thus, recruits two adapter complexes; TIRAP–MyD88, which drives the induction of inflammatory cytokines, and TRAM–TRIF, which induces type I IFN as well as inflammatory cytokines (Fig. 1). Notably, the TIRAP–MyD88 pathway is activated earlier than the TRAM–TRIF pathway. TIRAP contains a phosphatidylinositol 4,5-bisphosphate-binding domain required for retention to the plasma membrane. TLR4 engages with TIRAP on the cell surface and subsequently facilitates the delivery of MyD88 to initiate signaling, leading to NF-κB and MAPK activation (129) (Fig. 1). TLR4 is then internalized and trafficked to the endosome, where it promotes IRF3 activation and a second-phase NF-κB and MAPK activation via the TRAM–TRIF pathway (130, 131) (Fig. 1). TRAM is localized at the plasma membrane via its N-terminal myristoylation site and is phosphorylated by protein kinase Cε, which in turn triggers the TRIF pathway (132). TRAM phosphorylation might be required for endosomal trafficking of the TLR4 signaling complex.

TIRAP–MyD88 (TLR2 and TLR4 signaling) and TRAM–TRIF (TLR4 signaling) induce inflammatory responses via the recruitment of TRAF6, a member of TRAF family of proteins. In contrast, TRAF3 is recruited by MyD88 for TLR7 or TLR9 signaling and by TRIF in TLR3 signaling; in each of these cases, type I IFN is induced (133, 134). TRAF3 is also involved in RLR-mediated type I IFN induction (135). This suggests that TRAF3 functions as the general signal transducer for intracellular PRRs that specifically drives the type I IFN responses, whereas TRAF6 mainly mediates inflammatory responses in both cell-surface and intracellular PRR signaling pathways.

Activation of IL-1R-associated kinase and Tak1 during TLR signaling

MyD88 recruits the IL-1R-associated kinase (IRAK) family protein kinases IRAK4, IRAK1 and IRAK2 (Fig. 1B). IRAK4 is initially activated, and IRAK1 and IRAK2 are activated sequentially to induce rapid and sustained NF-κB activation, respectively, at least for TLR2 signaling (136). The activation of IRAKs results in TRAF6 activation. TRAF6 forms a complex with the E2 ubiquitin-conjugating enzyme complex Ubc13 and Uev1A to promote the synthesis of lysine 63-linked polyubiquitin chains to itself or to other substrates, which in turn activate transforming growth factor β-activated kinase 1 (TAK1). TAK1, in a complex with TAK1-binding protein (TAB1), TAB2 and TAB3, subsequently activates two distinct pathways involving the IκB kinase (IKK) complex or MAPK. The IKK complex, which is composed of the catalytic subunits IKKα and IKKβ and a regulatory subunit NF-κB essential modifier (NEMO, also known as IKKγ), catalyzes the phosphorylation of IκB proteins, triggering the degradation of IκBs and the subsequent nuclear translocation of NF-κB (2, 128). Genetic studies have revealed that Ubc13 has a critical role in MAPK activation rather than NF-κB activation, whereas TAK1 is required for both MAPK and NF-κB activation (137–139).

TRIF binds TRAF6 and receptor-interacting protein (RIP) 1 via distinct domains (Fig. 1). RIP-1 is inducibly ubiquitinated and facilitates interaction with TAK1. RIP-1 ubiquitination is abrogated in mice lacking TNFR-associated death domain (TRADD), which is an adapter. TRADD binds RIP-1 via its death domain and TRADD deficiency abrogated TLR3- and TLR4-mediated NF-κB and MAPK activation, at least in embryonic fibroblasts. Together, TRIF recruitment of TRAF6, RIP-1 and TRADD therefore facilitates TAK1 activation (140, 141).

Activation of IRFs during TLR signaling

As mentioned before, some TLRs involved in the recognition of viral PAMPs are able to trigger type I IFN via the activation of members of the IRF family. Of the nine members of the IRF family, IRF1, IRF3, IRF5, IRF7 and IRF8 are involved in TLR signaling. TLR3 and TLR4 stimulation recruits two non-canonical IKKs—TBK1 [also known as TRAF2-associated kinase (T2K) or NF-κB-activating kinase and IKKi (also known as IKKε)—to TRIF, which catalyzes the phosphorylation of IRF3 (Fig. 1F) (142, 143). Phosphorylated IRF3 forms a dimer and translocates to the nucleus to induce the expression of target genes including IFNβ. IRF7 is structurally the most similar to IRF3 and serves as the master regulator of type I IFN induction during TLR7 and TLR9 signaling in pDCs (Fig. 2A) (144). In pDCs, IRF7 is present in the cytoplasm, where it forms a signaling complex with MyD88, IRAK4, IRAK1, IKKα, TRAF6, TRAF3 and osteopontin (OPN) (81, 133, 134, 145–148). IRAK1 and IKKα promote phosphorylation and nuclear translocation of IRF7. In human pDCs, phosphatidylinositol 3 kinase (PI3K) is implicated in IRF7 phosphorylation (149). MyD88, IRAK4, TRAF6 and in some degree IKKα are required for both NF-κB and IRF7 activation, whereas IRAK1, TRAF3 and OPN selectively participate in IRF7 activation (Fig. 2A). IRF7 is highly expressed on pDCs, suggesting their ability to rapidly produce large amounts of type I IFN. The retention of the CpG-DNA–TLR9 signaling complex in the endosome offers an alternative mechanism for pDCs to produce robust type I IFN responses (150). IRF7 is also essential for type I IFN production in RLR signaling in many cells (144). In this case, TBK1 and IKKi rather than IRAK1

and IKKα are likely to mediate IRF7 phosphorylation and activation.

IRF5 and IRF1 are also recruited to MyD88 (151–153). IRF5 is phosphorylated and translocated to the nucleus where it binds to the IFN-stimulated response element of inflammatory cytokine genes (Fig. 2A). IRF1 is recruited to MyD88 in response to TLR9 in cDCs and, thus, induces IFNβ. IRF8 is a nuclear protein, which is highly expressed by pDCs and other DC populations. In IRF8-deficient mice, pDCs display a loss of TLR9-mediated induction of type I IFN and inflammatory cytokines accompanied by decreased NF-κB DNA binding (154). IRF8 is also required for the second, amplifying phase of IFN induction by viruses in both pDCs and other DCs (155). IRF8 physically interacts with IFN-inducible Ro52/TRIM21, a member of the tripartite motif family, which contain really interesting new gene (RING) finger (RNF), B box/coiled-coil and splA/ryanodine receptor (SPRY) domains and is an auto-antigen present in patients with systemic lupus erythematosus (SLE) or Sjögren's syndrome (156). Ro52 targets IRF8 for ubiquitination and potentiates the transcriptional activity of IRF8, which results in increased IL-12 p40 induction. Together, IRF8 may cooperate with other transcription factors such as IRF7 and NF-κB to facilitate target gene expression (Fig. 2).

RLR signaling pathways

RLR signaling results in activation of NF-κB, MAPK and IRFs for induction of type I IFN and inflammatory cytokines. IFNβ promoter stimulator 1 [IPS-1, also known as mitochondrial anti-viral signaling (MAVS), CARD adapter inducing IFNβ (Cardif) or virus-induced signaling adapter (VISA)] serves as an adapter for RIG-I and MDA5 (Fig. 2C) (157–160). IPS-1 contains an N-terminal CARD-like domain responsible for the interaction with RLRs and a transmembrane domain at the C-terminal end that is required for mitochondrial targeting as well as triggering anti-viral responses. The NS3/4A serine protease in HCV targets IPS-1 for cleavage at Cys-508, removing the transmembrane region, suggesting that HCV utilizes NS3/4A as a strategy to evade host anti-viral responses (159).

Downstream of IPS-1, there are the TBK1–IKKi and IKK complexes (Fig. 2C). IPS-1 recruits TRADD, which in turns assembles Fas-associated death domain protein (FADD) and RIP-1 (157, 161). Caspase-8 and caspase-10 are then recruited to FADD, where they are processed and activate NF-κB (162). TRADD simultaneously forms a complex with TRAF3, which induces TBK1–IKKi-dependent IRF3 activation. TBK1–IKKi also interacts with an RNA helicase, DEAD box polypeptide 3 X linked (DDX3), which enhances cytosolic RNA- and DNA-mediated IFNβ induction, although the function of this protein is largely unknown (163, 164). FADD is also implicated in IRF3 activation (165).

In addition to its role in DNA sensor signaling, STING is involved in RIG-I signaling (Fig. 2D) (84). STING interacts with RIG-I but not MDA5, and cells lacking STING showed attenuated type I IFN induction following infection with VSV. In contrast, responses to TLR ligands were unimpaired by STING deficiency (84). More recently, mediator of IRF3 activation (MITA) was identified in a screen; it can activate an IFN promoter and was found to be identical to STING (166). Unlike STING, that was initially shown to reside in the ER, MITA was shown to be localized at the outer membrane of mitochondria, where it interacts with IPS-1 and recruits IRF3. The mechanistic basis for differences regarding cellular localization of STING and MITA is unclear.

TRIM25 participates in the regulation of RIG-I signaling. TRIM25 directly binds to RIG-I and promotes Lys-63-linked ubiquitination of the CARD of RIG-I, which facilitates the recruitment of IPS-1 to activate signaling. TRIM25-null cells consistently display a loss of RIG-I ubiquitination as well as impaired anti-viral responses (167).

The dectin-1 signaling pathway

Engagement of β-glucan on pathogens such as fungi and yeast to host dectin-1 induces phagocytosis and ROS production and recruits the spleen tyrosine kinase (Syk) via the ITAM motif of dectin-1 (168, 169). Syk activates downstream signaling through CARD9, which then recruits Bcl-10–MALT1 (mucosa-associated lymphoid tissue 1) and activates NF-κB (170–172) (Fig. 3B). This pathway triggers preferential differentiation of T cells into T_h17 cells, which is required for host defense against fungal infection (173–175). In addition, CARD9 is implicated in TLR, NOD1 and NOD2-mediated MAPK activation. These findings suggest a role of CARD9 as an integral component of TLR, dectin-1, NOD1 and NOD2 signaling (Fig. 3) (170–172).

The NOD1 and NOD2 signaling pathways

NOD1 and NOD2 signaling leads to NF-κB and MAPK activation (Fig. 3A). PAMP stimulation induces self-oligomerization of NOD1 and NOD2, allowing the recruitment of the CARD-containing serine/threonine kinase RICK (RIP-like interacting caspase-like apoptosis regulatory protein kinase, also known as RIP-2). RICK subsequently activates TAK1 through Lys63-linked ubiquitination, which finally activates NF-κB and MAPK (176–178). The activation of MAPK via NOD2 requires CARD9 (Fig. 3A) (172).

Negative regulation of PRR responses

It appears that the innate immune responses triggered by PRR are critical elements in host defense; however, aberrant activation of the PRR response is tightly associated with various diseases including inflammation, autoimmune diseases and tumor development. Negative regulation of PRR responses is therefore crucial to maintain homeostasis, and indeed, there are multiple mechanisms that suppress deleterious induction of cytokines by limiting PRR responses. These include degradation and sequestration of signaling molecules, inhibition of transcription and inhibitory signals from certain receptors that antagonize PRR signaling. These include radioprotective 105-kDa (RP105), ST2L, single immunoglobulin IL-1R-related protein (SIGIRR), two RING fingers and double RING finger-linked 3A (Triad3A), suppressor of cytokine signaling (SOCS) 1, sterile α and HEAT/Armadillo motif (SARM), IRAK-M, splicing variants of IRAK1, IRAK2 and MyD88, TRAF4, β-arrestins, FLN29, A20, peptidylprolyl *cis*/*trans*-isomerase, NIMA-interacting 1 (PIN-1), suppressor of IKK-ε (SIKE), CYLD, PI3K, IRF4 and activation

transcription factor (ATF) 3. As the negative regulation of TLR signaling has been reviewed elsewhere (128, 179), we describe more recent findings here.

Two tyrosine phosphatases—src homology 2 domain-containing tyrosine phosphatase (SHP) 1 and SHP2—were demonstrated to negatively regulate the TLR and RLR signaling pathways. SHP1 reduces TLR-mediated inflammatory cytokine induction by suppressing NF-κB and MAPK activation (180); however, it simultaneously promotes type I IFN induction. SHP2 targets TBK1 to suppress the production of inflammatory cytokines and type I IFN induced by TLR3 (181).

RLR signaling is negatively regulated by multiple mechanisms. The E3 ubiquitin ligase, RNF125, promotes ubiquitination and proteasomal degradation of RIG-I, whereas the dihydroacetone kinase (DAK) binds to MDA5 and suppresses downstream signaling (182, 183). The TRAF3-mediated antiviral response is negatively regulated by deubiquitinating enzyme A (DUBA), which cleaves Lys-63-linked polyubiquitin chains and causes TRAF3 to dissociate from TBK1–IKKi (184).

NLRX1, a member of the NLR family, contains a mitochondrion-targeting sequence and is localized to the outer membrane of the mitochondria. NLRX1 disrupts interactions between RLRs and IPS-1, resulting in reduced NF-κB and IRF activation (185); however, NLRX1 is also implicated in enhancing ROS induction and activation of NF-κB and JNK (186). Mitochondrial localization is also required for this NLRX1-mediated enhancement. NLRX1 may therefore prevent interactions between IPS-1 and RLRs in the steady state; however, in response to infection, it may be converted to a positive regulator for inflammation by inducing ROS.

Recent studies have shown that ATG16L1, which is implicated in Crohn's disease (187), negatively controls LPS-induced inflammasome activation (188). ATG16L1-deficient cells display severe impairment of autophagosome formation and degradation of long-lived proteins, and macrophages derived from these mice exhibit increased caspase-1 activation and a high amount of IL-1β and IL-18 production in response to LPS. ATG16L1-deficient mice are highly susceptible to dextran sulfate sodium-induced acute colitis, which is blocked by anti-IL-1β and anti-IL-18 antibodies; moreover, Paneth cells derived from ATG16L1-deficient mice show increased expression of genes involved in responses to intestinal injury, and patients who have Crohn's disease and bear a ATG16L1 mutation exhibit similar Paneth cell abnormalities (189). ATG16L1 is, thus, essential for the suppression of intestinal inflammation.

Activation of transcription factors is also negatively regulated. Ro52 binds to IRF3 to promote ubiquitination and degradation, whereas it increases IRF8 activity (190). PDLIM2 [PSD95, DlgA, zo-1 (PDZ) and Lin11, Isl-1, Mec-3 (LIM) domain 2] has roles in suppressing the induction of certain NF-κB-dependent genes by destroying nuclear RelA (191). Mitogen- and stress-activated kinase (MSK) 1 and MSK2, which are downstream kinases of p38 and ERK1/2, phosphorylate cyclic adenosine 3′,5′-monophosphate response element-binding protein (CREB) and ATF to induce the anti-inflammatory cytokine IL-10 and dual specificity phosphatase 1 (DUSP1) to limit inflammation (192).

TLR-mediated responses are suppressed by signals from other receptors. TAM (Tyro3, Axl, Mer) receptors, which are receptor tyrosine kinases whose inactivation leads to autoimmunity, activate signal transducer and activator of transcription 1 (STAT1) to induce SOCS1 and SOCS3, to block TLR signaling (193). In human pDCs, ITAM-mediated, B cell antigen receptor (BCR)-like signaling pathways potently suppress TLR7- and TLR9-mediated type I IFN induction (48).

The contributions of PRRs to shaping adaptive immune responses

Innate immune recognition of PAMPs is an essential element to instruct adaptive immune responses (4). Different classes of PRRs share PAMPs for recognition in different cell types or the same cell types. For example, RNA viruses are sensed by TLR7 on pDCs and RLRs in other cell types. DNA viruses are sensed by TLR9 and the cytosolic DNA sensor, and flagellin is sensed by TLR5 and IPAF–NAIP5. It is, however, still unclear how these PRRs contribute to the generation of adaptive immune responses.

LCMV is an ambisense ssRNA virus that is known to provoke $CD8^+$ T cell activation in a type I IFN-dependent manner. Generation of virus-specific $CD8^+$ T cells following LCMV infection was abrogated in the absence of TLR signaling, but it was normal in mice deficient for RLR signaling (194). In the absence of TLR signaling, the production of type I IFN was impaired. The sources of IFNα following LCMV infection are likely to be pDCs, suggesting that TLR recognition of LCMV by pDCs plays an important role in the efficient development of anti-viral adaptive immune responses.

RNA derived from influenza A virus is recognized by TLR7 or RIG-I in a cell-type-specific manner. Recognition of this virus by pDCs preferentially relies on TLR7, whereas other cell types, such as alveolar macrophages, cDCs and fibroblasts, utilize RIG-I (195, 196); however, in the lung, both pathways are important for robust induction of type I IFN. In the *in vivo* situation, the production of antibodies specific to influenza A virus and $CD4^+$ T cell responses following intranasal infection were controlled by TLRs rather than RLRs. Accordingly, vaccination with inactivated influenza A virus fails to protect against the resulting infection in the absence of TLR signaling. This suggests that TLRs rather than RLRs contribute to the induction of effective anti-viral adaptive immune responses (195); however, mice deficient for either the TLR or the RLR pathway developed normal antigen-specific $CD8^+$ T cell responses against live influenza A virus, suggesting the existence of TLR-independent and RLR-independent mechanisms with regards to $CD8^+$ T cell responses (195). Similarly, TLR-independent and RLR-independent pathways are also suggested in the case of RSV infection (197), although the pathways that govern this form of T cell activation are uncertain.

On the other hand, a contribution of the RLR pathway has also been reported. Poly IC has an adjuvant property that enhances humoral and cell-mediated immunity as well as NK-cell-dependent anti-tumor properties poly IC is recognized by TLR3, RIG-I and MDA5. Poly IC-enhanced antigen-specific antibody production, the expansion of antigen-specific $CD8^+$ T cells and the production of IFNγ were completely lost in mice lacking both TLR3 and RLR signaling pathways,

indicating that combinational recognition by TLRs and RLRs is essential for the robust innate and adaptive immune responses (198). The TLR3-dependent pathway was shown to be critical for poly IC-dependent anti-tumor activity (199). This activation requires DC–NK cell interaction rather than cytokine production.

DNA vaccines possess sequences encoding antigen as well as elements that initiate innate immune responses, probably through the cytosolic DNA sensor and TLR9. Importantly, the induction of B and T cell responses by DNA vaccination occurs via TLR-independent, RLR-independent and DAI-independent pathways, but requires the presence of TBK1 and type I IFN. Notably, TBK1 functions in both immune and non-immune cells, and activation of both types of cells is required to elicit robust antibody production and T cell activation (83).

NOD1 stimulation alone has the capacity to drive T_h2 responses; however, when given with TLR ligands, NOD1 signaling enhances T_h1, T_h2 and T_h17 responses, suggesting that NOD1 signaling potentiates the adaptive immune responses that are mediated by TLRs (200). NOD2-deficient mice had defects in their ability to mount antibody responses when MDP was given as an adjuvant (93). NOD2 mutations are linked to Crohn's disease, and the IL-23-derived and IL-1β-derived T_h17 responses induced by NOD2 ligand are abrogated in cells from patients who carry NOD2 mutations (201).

Alum, which is sensed by NALP3, is widely used as an adjuvant in human, and is known to trigger local recruitment of monocytes and migration into the draining lymph node to prime T cells as well as promote migration of myeloid cells to the spleen to activate antigen-specific B cells. Alum can increase the production of IL-1β via the NALP3 inflammasome as well as via phagocytosis. The production of antigen-specific antibodies following immunization of antigen given in alum was abrogated in mice lacking a component of the NALP3 inflammasome, suggesting the importance of the NALP3 inflammasome in shaping adaptive immune responses (109, 202, 203); however, there is another report showing an indispensable role of the NALP3 inflammasome in alum-induced boosting of antibody production (204). These differences may come from the dose of antigen and adjuvant used, the type of antigen, the route of immunization and immunization protocol.

Triggering of autoimmunity by endogenous nucleic acids

Elevated type I IFN production owing to defective clearance of self-derived nucleic acids is tightly linked to autoimmune diseases such as SLE (48, 205). For example, deficiency or mutations in genes encoding an extracellular DNase I are associated with lupus-like syndrome in mice and humans (206, 207); moreover, mice lacking lysosomal DNase II show accumulation of incompletely digested DNA, which causes TLR-independent type I IFN and inflammatory responses, which are linked to autoimmune symptoms like chronic polyarthritis (208–210). Mice with mutations in Flap endonuclease 1 (FEN1) also display increased amounts of undigested DNA within apoptotic cells and are predisposed to autoimmunity, chronic inflammation and cancer (211); moreover, mutations in 3′-repair exonuclease 1 (Trex1), a 3′–5′ DNA exonuclease, are found in people with SLE and Aicardi–Goutieres syndrome associated with elevated type I IFN (212, 213), and Trex1 is suggested to negatively control innate responses to cytoplasmic dsDNA (214). Collectively, these findings suggest that the intrinsic responses to accumulated cellular DNA may define several autoimmune diseases, probably through a cytosolic DNA sensor; however, *in vivo* situations whereby the accumulation of undigested self-DNA within apoptotic cells is triggered are as-yet undefined.

The other platform for initiation or amplification of autoimmunity is TLR recognition of self-derived nucleic acids. Self-DNA, which does not activate TLR9 in normal conditions, is converted to stimulate pDCs in certain pathogenic conditions, triggering autoimmunity. The anti-microbial peptide LL37 (a cathelicidin), which is produced by neutrophils and keratinocytes and highly expressed in skin lesions in psoriasis, forms aggregates with self-DNA that is derived from necrotic cells, and these LL37–self-DNA aggregates are endocytosed and retained in early endosomes in pDCs and, in turn, engage with TLR9 to promote type I IFN production (215). Similarly, anti-DNA antibodies produced by autoreactive B cells in SLE bind self-DNA and induce type I IFN by pDCs via a cooperation of TLR9 and FcγRIIa (216). HMGB1, which is released from necrotic cells or cells stimulated with TLR ligands, enhances induction of type I IFN by DNA-containing immune complexes after binding DNA. The HMGB1–DNA complex binds to a receptor for advanced glycation end products expressed on pDCs, which facilitates the engagement of the DNA with TLR9 in endosomes (217).

Similarly, immune complexes can also promote proliferation of autoreactive B cells through dual recognition by the BCR and TLR9 (218). Collectively, these findings suggest that endogenous molecules can function as a trigger for autoimmunity by facilitating TLR9 signaling in pDCs and/or B cells. The other potential factors that facilitate autoimmunity are cathepsins, which are implicated in TLR-triggering inflammatory responses (219). Cathepsin K, which is expressed by osteoclasts, is reportedly able to enhance TLR9-mediated inflammation in DCs, a process that is linked to autoimmunity in a mouse model of experimental arthritis (220).

Likewise, self-RNA also contributes to autoimmunity by activating TLR7. Small nuclear ribonucleoprotein complexed with auto-antibody is taken up in pDCs and induces TLR7-triggered type I IFN and inflammatory responses (221). This complex can also activate auto-reactive B cells via dual recognition by the BCR and TLR7 (221, 222); moreover, duplication of the TLR7 gene is found in mice that are hyperreactive to TLR7 ligands and display autoimmune nephritis (223, 224).

Self-RNA is also recognized by RLRs. Viral RNA stimulates 2′5′ oligoadenylate synthetase to promote activation of an endonuclease, RNaseL, which subsequently cleaves host cellular RNA to create small RNA species (225). These RNA serve as the ligand for RIG-I and MDA5. This may function as a host defense mechanism against viruses by amplifying type I IFN responses; however, it is not clear whether the RLR pathway contributes to autoimmune diseases.

Future perspectives

The discovery of the transmembrane TLRs and cytosolic sensing systems such as RLRs, NLRs and the DNA sensors has revealed that the innate immune system possesses multiple recognition mechanisms in different cellular compartments (e.g. plasma membrane, endosome, lysosome, cytoplasm) and in different cell types (e.g. TLR7–TLR9 in pDCs versus RLRs in cDCs). It appears that each PRR signaling pathway plays an indispensable role in the elimination of pathogens or to maintain tolerance because mutations of PRRs or their signaling molecules (e.g. TLR2, TLR3, TLR4, TLR5, MDA5, NALP1, NALP3, NALP7, pyrin, NOD1, NOD2, MyD88, IRAK4, IRF5, UNC93B or ATG16L1) are linked to many inflammatory diseases, immunodeficiencies and autoimmune diseases in humans (2, 187, 226–230).

A single PAMP is sometimes recognized by distinct PRRs, which synergistically induce inflammatory responses and instruct adaptive immune responses. Understanding of the complexity of PRRs, with respect to the coordinated control of both innate and adaptive immune responses, is thus required for future development of therapeutic drugs that effectively or qualitatively control immune-associated diseases, including infectious diseases, inflammatory diseases, allergies, autoimmune diseases and cancer.

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Abbreviations

Alum	aluminum salt
ATF	activation transcription factor
ASC	apoptosis-associated speck-like protein containing a CARD
Bcl	B cell lymphoma
BCR	B cell antigen receptor
BIR	baculovirus inhibitor of apoptosis protein repeat
Birc1e	BIR-containing 1e
CARD	caspase recruitment domain
CARDINAL	CARD8, DACAR, NDPP1 and TUCAN
cDC	conventional dendritic cell
CpG	cytidine–phosphate–guanosine
DAI	DNA-dependent activator of IRF
DC	dendritic cell
dsDNA	double-stranded DNA
dsRNA	double-stranded RNA
EMCV	encephalomyocarditis virus
ER	endoplasmic reticulum
ERK1/2	extracellular signal-regulated kinase 1/2
FADD	Fas-associated death domain protein
GPI	glycosylphosphatidylinositol
HCV	hepatitis C virus
HMGB1	high-mobility group box 1
HSP	heat shock protein
HSV	herpes simplex virus
hToll	human homologue of Toll
Hz	hemozoin
iE-DAP	g-D-glutamyl-meso-diaminopimelic acid
IκB	inhibitor of κB
IKK	IκB kinase
IPAF	IL-1β-converting enzyme protease-activating factor
IPS-1	IFNβ promoter stimulator-1
IRAK	IL-1R-associated kinase
ITAM	immunoreceptor tyrosine-based activation motif
IRF	IFN regulatory factor
JNK	c-jun N-terminal kinase
LBP	LPS-binding protein
LCMV	lymphocytic choriomeningitis virus
LC3	light chain 3
LGP2	laboratory of genetics and physiology 2
LPDC	lamina propria dendritic cells
LRR	leucine-rich repeats
LT	lethal toxin
LTA	lipoteichoic acid
MALT1	mucosa-associated lymphoid tissue 1
MAPK	mitogen-activated protein kinase
MCMV	murine cytomegalovirus
MDA5	melanoma differentiation associated gene 5
MITA	mediator of IRF3 activation
mRNA	messenger RNA
MSK	mitogen- and stress-activated kinase
MyD88	myeloid differentiation primary response gene 88
MDP	muramyl dipeptide
NAIP	neuronal apoptosis inhibitory protein
NALP	NACHT-LRR-PYD-containing protein
NEMO	NF-κB essential modifier
NF-κB	nuclear factor kappa B
NOD	nucleotide-binding oligomerization domain
NLR	NOD-like receptor
ODNs	oligodeoxynucleotides
OPN	osteopontin
PAMPs	pathogen-associated molecular patterns
pDC	plasmacytoid dendritic cell
PGN	peptidoglycan
PI3K	phosphatidylinositol 3 kinase
PRR	pattern recognition receptor
poly IC	polyinosinic-polycytidylic acid
RD	repressor domain
RICK	RIP-like interacting caspase-like apoptosis regulatory protein kinase
RIG-I	retinoic acid-inducible gene-I
RING	really interesting new gene
RIP	receptor-interacting protein
RLR	RIG-I-like receptor
RNF	RING finger
ROS	reactive oxygen species
RSV	respiratory syncytial virus
SGT1	suppressor of G_2 allele of S-phase kinase-associated protein 1
SHP	src homology 2 domain-containing tyrosine phosphatase
siRNA	small interfering RNA
SLE	systemic lupus erythematosus
Syk	spleen tyrosine kinase
SOCS	suppressor of cytokine signaling
ssRNA	single-stranded RNA
STING	stimulator of IFN genes
TAB	TAK1-binding protein
TAK1	transforming growth factor β-activated kinase 1
TANK	TNFR-associated factor family member-associated nuclear factor κB activator
TBK1	TANK binding kinase 1
TICAM	TIR domain-containing adapter molecule
TIR	Toll/IL-1R
TIRAP	TIR domain-containing adapter protein
TLR	Toll-like receptor
TRADD	TNFR-associated death domain
TRAF	TNFR-associated factor
TRAM	TRIF-related adapter molecule
TRAP	translocon-associated protein
Trex1	3′-repair exonuclease 1
TRIF	TIR-containing adapter inducing IFNβ

VSV	vesicular stomatitis virus
WNV	West Nile virus

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